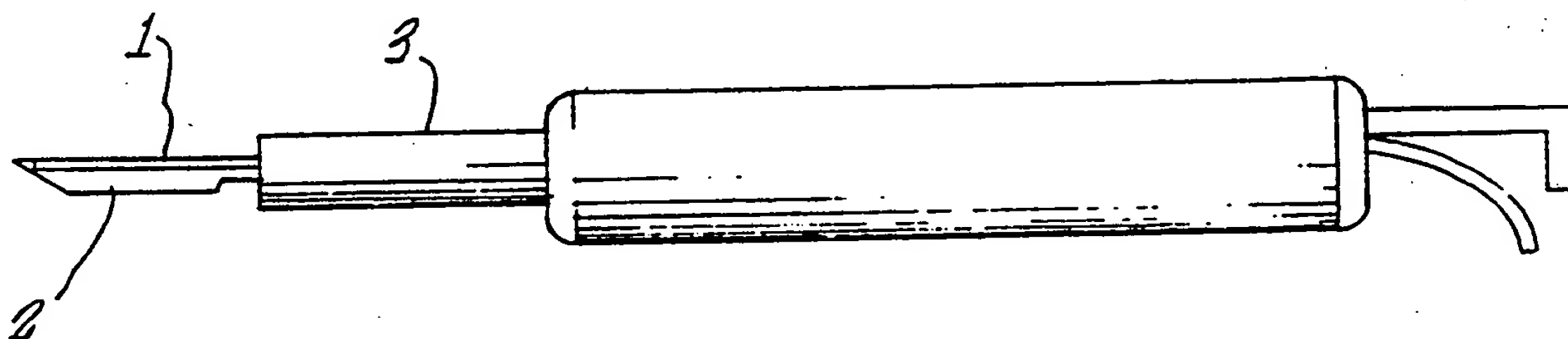


## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b>  A61B 5/00	<b>A1</b>	<b>(11) International Publication Number:</b> WO 92/17108  <b>(43) International Publication Date:</b> 15 October 1992 (15.10.92)
<b>(21) International Application Number:</b> PCT/US92/02042 <b>(22) International Filing Date:</b> 12 March 1992 (12.03.92)  <b>(30) Priority data:</b> 679,766                      3 April 1991 (03.04.91)                      US  <b>(71) Applicant:</b> CEDARS-SINAI MEDICAL CENTER [US/US]; 8700 Beverly Boulevard, Los Angeles, CA 90048 (US).  <b>(72) Inventors:</b> VARI, Sandor, George ; 16547 Vanowen Street, Apt. #104, Van Nuys, CA 91406 (US). PAPAOGLOU, Theodore ; 3 Kalosgourou Street, GR-111 41 Athens (GR). GRUNDFEST, Warren, Scott ; 8272 Skyline Drive, Los Angeles, CA 90046 (US).		<b>(74) Agents:</b> HAAKE, Deborah, L. et al.; 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US).  <b>(81) Designated State:</b> JP.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PHOTSENSITIZER ENHANCED FLUORESCENCE BASED BIOPSY NEEDLE  <div data-bbox="373 1793 1989 2144"></div> <b>(57) Abstract</b>  A method and apparatus is disclosed for the instant intraoperative detection and biopsy of metastatic cancer using fluorescence spectroscopy. A photosensitizing agent selectively retained by cancerous tissue is administered prior to surgery. A fiberoptic probe (1) integrated with a biopsy device (2) illuminates the examined tissue and causes fluorescence which is recorded by a spectrograph and plotted as a spectral curve. The intensity ratio (S1/S2) for the fluorescence from the photosensitizing agent (S1) and autofluorescence (S2) for the examined tissue is compared with the intensity ratio at the same wavelengths for primary tumor and normal tissue. Tissue that displays an intensity ratio different from that of normal tissue can immediately be analyzed for the depth of tumor involvement and then excised for histological examination using the biopsy device (2).		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	RU	Russian Federation
CG	Congo	KP	Democratic People's Republic of Korea	SD	Sudan
CH	Switzerland	KR	Republic of Korea	SE	Sweden
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
DE	Germany	MC	Monaco	TG	Togo
DK	Denmark			US	United States of America

DESCRIPTION

## Photosensitizer Enhanced Fluorescence Based Biopsy Needle

Field of the Invention

The present invention relates generally to a method and apparatus for the instant intraoperative detection and biopsy of metastatic cancer using fluorescence spectroscopy.

Background

Perhaps no disease known to modern civilization is viewed with as much general fear as cancer. Heart attacks and strokes tend to be thought of as natural hazards of age, and either a normal end to a satisfactorily long life or, when they occur in middle-age, the wages of a sedentary lifestyle. In contrast, cancer is thought of as an unpredictable disease that strikes indiscriminately at rich and poor, fat and thin, old and young, as if it owed nothing to external causes. J. Cairns, Mutation, Selection and Cancer, Nature, Vol. 255, pp. 197-200 (1975).

Each year in the United States, approximately 600,000 new cases of cancer are diagnosed; and one out of every five people in this country will die from cancer, or from complications associated with its treatment. However, most of these cancer patients are not killed by their primary tumor. They succumb instead to metastasis: multiple, widespread tumor colonies established by malignant cells that detach themselves from the original tumor and travel through the body, often to distant sites. G. Nicholson, Experimental Tumor Metastasis: Characteristics in Organ Specificity, Bioscience, Vol. 28, No. 7, pp. 441-47 (July, 1978). If a primary tumor is detected early enough, it can usually be eliminated by surgery, radiation, chemotherapy or photodynamic therapy,

or some combination of these treatments. Unfortunately, the metastatic colonies are harder to detect and eliminate because they often are not visible to the unaided eye, being microscopic in size or hidden within tissues or  
5 organs. This difficulty in detecting and eradicating metastasis enhances cancer's image as a fleeting and unpredictable disease.

Presently, primary tumor detection is accomplished by x-ray, ultrasonography, nuclear magnetic resonance (NMR),  
10 positron emission tomography (PET), chemical laboratory analysis and biopsy. However, metastatic dissemination from the primary tumor often is impossible to detect with these methods. As a result, there is a definite need for an accurate and sensitive technique to detect and sample  
15 these elusive metastatic colonies.

Every diagnosis of cancer must be documented by a definitive biopsy. In addition, biopsy is a crucial technique in the management of cancer. Since different types of neoplasms have their own responses to the various  
20 modalities of therapy, a histological diagnosis is imperative in planning the appropriate management of malignant disease. Moreover, biopsy provides the pathologist with adequate samples of tumor. In addition to the biopsy, an immediate frozen section during intra-  
25 operative procedures is often necessary for final diagnosis of malignancy. However, frozen section histology is both time consuming and expensive. Performed during the operation, this procedure requires the surgeon to halt the surgery midstream until the pathology of the  
30 suspected cancerous tissue is determined. Once determined, the operation can then be resumed. Consequently, the availability of a device that provides an instant indication of metastasis, as well as a simultaneous biopsy sample of the suspected tissue during  
35 the operative procedure would satisfy the need for a definitive biopsy and eliminate the disadvantages of frozen tissue histology.

With this in mind, it has been known for more than sixty years that some porphyrins are selectively retained by neoplastic tissue. A. Policard, Etudes Sur Les Aspects Offerts Par De Tumeurs Experimentales Examinees à La  
 5 Lumiere De Woods, C.R. Séanc Soc. Biol. 91:1423-4 (1924). These same porphyrins also emit a characteristic dual-peaked red fluorescence after being exposed to light containing the appropriate wavelength to excite fluorescence. Although any fluorescent agent selectively  
 10 retained by cancerous tissue can be used, Lipson in 1961, used these two properties of porphyrins to develop a primary tumor detection system. Lipson also introduced hematoporphyrin derivative (HPD) which demonstrated better tumor-localizing properties. R.L. Lipson, et al., The Use  
 15 of a Derivative of Hematoporphyrin in Tumor Detection, J. Nat. Cancer Inst. 26:1-11 (1961). Dougherty then took advantage of HPD's photosensitizing properties to eradicate tumors, opening the door to HPD's use as a therapeutic cancer modality. Since that time, HPD's  
 20 primary importance in the cancer arena has been as a potential cancer treatment, and extensive investigation has been done to refine its use. T. Dougherty, C.R.C. Critical Review in Oncology/Hematology, S. David, E.D. (C.R.C. Press, Florida, 1984).

25       Termed, "Hematoporphyrin Derivative Photodynamic Therapy," HPD's mechanism in cancer therapy is based on its affinity for malignant tumors relative to other tissues. When injected intravenously, HPD localizes at  
 30 higher levels in malignant tumor tissues than in normal tissues. The HPD is then activated by light to catalyze the production of singlet oxygen from available triplet oxygen. Although the exact mechanism of necrosis is unclear, it has been suggested that the reactive singlet oxygen oxidizes unsaturated carbon-carbon bonds in amino  
 35 acids and fatty acids. The ensuing loss of the structural integrity of cellular macromolecules results in cytocidal effects and tumor necrosis. Li, et al., Application of

HPD and Laser-Induced Photo Dynamical Reaction in the Treatment of Lung Cancer, Laser in Surgery and Medicine, 4:31-7 (1984).

This use of HPD in photodynamic cancer treatment is a very exiting and rapidly developing possibility. However, a complete cure for cancer is impossible without specific detection and ablation of those cancer cells that have disseminated throughout the organism via the lymphatic or circulatory system. The present invention deals with this problem of metastasis and facilitates a potential cancer cure by utilizing HPD's tumor-seeking properties and fluorescence to instantly detect and simultaneously biopsy cancerous tissue undetected by conventional methods.

Although much of the research involving HPD has been with regard to cancer treatment, several investigators have looked at HPD's tumor detection capabilities. However, the goal of this detection work has been to use surface fluorescence to localize carcinoma in situ which by definition has not penetrated the basement membrane, and thus is not metastasizing. In contrast, the present invention is capable of detecting and biopsying metastatic sites, as well as determining tumor depth and size. In fact, the present invention is based in part on the discovery that metastatic sites which generally are undetected by conventional diagnostic methods produce a fluorescence spectra different from that of primary tumor and normal tissue. The photosensitizer enhanced fluorescence spectra of metastatic sites are consistently and significantly higher than the fluorescence spectra of both the primary tumor and normal tissue.

Profio, et al. described a fluorescence bronchoscopy system for localizing small lung tumors and carcinomas in situ by HPD fluorescence. E. Profio, Fluorescence Bronchoscopy for Localization of Carcinoma In Situ, Med. Phys. 10 (1), pp. 35-39 (Jan./Feb. 1983). Ankerst, Montan and Svanberg each studied HPD laser-induced fluorescence

in normal and tumor rat tissue to determine optimal HPD surface fluorescence for tumor detection. J. Ankerst, et al., Laser-Induced Fluorescence Studies of Hematoporphyrin Derivative (HPD) in Normal and Tumor Tissue of Rat, Applied Spectroscopy, Vol. 38, No. 6, pp. 890-96 (1984); S. Montan, Multicolor Imaging and Contrast Enhancement in Cancer-Tumor Localization Using Laser-Induced Fluorescence in Hematoporphyrin-Derivative-Bearing Tissue, Optics Letters, Vol. 10, No. 2, pp. 56-8 (February, 1985); and K. Svanberg, Fluorescence Studies of Hematoporphyrin Derivative in Normal and Malignant Rat Tissue, Cancer Research, 46:3806-808 (August, 1986). Kato, et al. described four different bronchoscopic fluorescence detection systems that have facilitated the localization of HPD-labelled squamous cell carcinoma of the trachea and the central bronchi. H. Kato, et al., Early Detection of Lung Cancer by Means of Hematoporphyrin Derivative Fluorescence and Laser Photoradiation, Clinics in Chest Medicine, Vol. 6, No. 2, pp. 337-53 (June, 1985).

Others have attempted to utilize HPD's tumor seeking properties to detect cancer, but have failed. This failure was apparently due to an intrinsic abundance of free porphyrins in cancerous tissue and HPD's uptake in normal tissue. It therefore was concluded that HPD did not provide a good in vivo technique for detection. The present invention, however, overcomes these failures, and is capable of detecting cancer in situ as well as difficult to find metastasis. Moreover, once detected, the present invention also allows for the immediate biopsy of metastatic sites.

In U.S. Pat. No. 4,930,516 to R. R. Alfano, there is described a method and apparatus for detecting the presence of tumors in situ using the native visible luminescence of the cell. The invention is based exclusively on the intrinsic fluorescence of the cell produced by native flavins and porphyrins found in abundance in subcellular organelles.



Summary of the Invention

The present invention for the instant intraoperative detection and biopsy of metastatic cancer involves, according to one embodiment of the invention, administering prior to surgery, a photosensitizing agent selectively retained by cancerous tissue. The region to be examined is then illuminated with a beam of monochromatic light and/or incoherent light filtered to a specific wavelength from the fiber optic portion of the fiber optic biopsy probe, and the emitted fluorescence is recorded by a spectrograph and plotted as a spectral curve. The intensity ratio ( $S_1/S_2$ ) of the photosensitizer induced fluorescence ( $S_1$ ) and autofluorescence ( $S_2$ ) for the examined tissue is compared with the intensity ratio at the same wavelengths for primary tumor and normal tissue. All tissue that displays a fluorescence pattern different from normal can immediately be examined for tumor depth, excised using the biopsy portion of the fiber optic biopsy probe and then subjected to histological examination.

The apparatus for the present invention, according to one embodiment, includes a light source, a spectrograph, a video camera, a digitizer, a computer, a display means for measuring and comparing the intensity of the emitted light over a plurality of wavelengths, and a biopsy device.

The apparatus of the present invention according to another embodiment, includes a light source, optical filters, a photo detector, a display means for measuring the emitted light at different wavelengths and a biopsy device. It is to be understood however, that other embodiments may be utilized and that structural changes may be made without departing from the scope of the invention. The following detailed description is therefore, not to be taken in a limiting sense, and the



scope of the present invention is best defined by the appended claims.

It is a general object of the present invention to provide an apparatus and method for the instant  
5 intraoperative detection of metastasis not visible to the naked eye.

It is another object of the present invention to provide an apparatus and method for the instant intraoperative detection of metastasis undetected by  
10 conventional diagnostic methods employed to detect primary tumors.

It is still another object of the present invention to provide an apparatus and method for the instant intraoperative detection of metastasis using fluorescence  
15 spectroscopy.

It is a further object of the present invention to provide an apparatus and method for the instant determination of the extent of metastatic involvement in an affected tissue.

20 It is still further an object of the present invention to provide an apparatus and method for immediately excising upon detection the suspected metastatic tissue.

It is also an object of the present invention to  
25 eliminate the time and expense incurred by frozen section histology.

These and other objects will become readily apparent to those skilled in the art from the following description and the appended claims.

### 30 Brief Description of the Drawings

FIG. 1 is a schematic diagram of an experimental optical biopsy probe.

FIG. 2 is a fluorescence spectra of rat malignant tissue containing Photofrin II and the autofluorescence  
35 spectra of rat tissue over time.

FIG. 3 is a graphic distribution of the fluorescence intensity ratio of Photofrin II to autofluorescence for various Photofrin II doses in different rat tissues.

FIG. 4 is a graphic distribution of the fluorescence intensity ratio of Photofrin II to autofluorescence for the accumulation of Photofrin II in specific rat tissues over time.

FIG. 5 is a graphic distribution of the fluorescence intensity ratio of Photofrin II to autofluorescence for different doses of Photofrin II in specific rat tissues forty-eight hours after administration.

#### Detailed Description of the Invention

The present invention is directed to a method and apparatus for the instant intraoperative detection and biopsy of metastatic cancer using fluorescence spectroscopy.

The experimental arrangement used to measure the fluorescence spectra during abdominal exploration included a Helium-cadmium laser operating at 442 nm as an excitation source. A hand held 400 micron core diameter fiber was directed at the tissue producing a low power (17 mw) illumination. Using the same fiber, reflected and emitted light was returned to the input of a SPEX 500 spectrometer. The spectrometer output was directed to a Silicon Diode Array (Model A 1420 B, EG&G) coupled into an optical multi-channel analyzer (OMA III EG&G). Signals from the OMA were then displayed on a screen for immediate examination. A series of normal spectra were recorded from the left medial thigh muscles for baseline. The fluorescence intensities at 540 nm (auto- fluorescence) and 630 nm (Photofrin II) were simultaneously monitored. By using the ratio of these intensities ( $I_{630 \text{ nm}}/I_{540 \text{ nm}}$ ), a relative value could be assigned to each area studied. An increased value of the ratio of intensities ( $I_{630 \text{ nm}}/I_{540 \text{ nm}}$ ) signaled a possible metastatic site. Tissues displaying such an increased ratio of intensities were removed using

a biopsy device integrated with the fiberoptic probe and subjected to histological examination.

In detecting the presence of cancerous tissue in accordance with the invention, the ratios of two probe  
5 signals S1 and S2 ( $S1/S2$ ) are first determined for a known non-cancerous region. S1 represents the fluorescence intensity at 630 nm (Photofrin II); and S2 represents the fluorescence intensity at 540 nm (auto-fluorescence). An increased value of the  $S1/S2$  ratio signals a possible  
10 metastatic site.

Alternatively, the light source may comprise any device emitting substantially monochromatic light and/or incoherent light filtered to a specific wavelength. The fiber optic probe is composed of either multiple or single  
15 fiber arrays, the core diameter of which is preferably about 400 microns. Integrated with the fiber optic probe is a biopsy needle (Baxter, Tri-Cut® or any conventional biopsy needle) for instant determination of tumor depth and immediate tissue removal. The biopsy needle can be  
20 calibrated (inches, millimeters) to visually determine the depth of tumor involvement in the affected tissue. A schematic diagram of an experimental optical biopsy probe is illustrated in FIG. 1. The optical probe 1 can be integrated in any manner to the top surface of the biopsy  
25 blade 2, and then housed in a probe casing 3. However, it is understood that other embodiments may be utilized and structural changes made to the optical biopsy probe without departing from the scope of the invention.

The fluorescence emitted from cancerous and normal  
30 tissues of twenty male Lobund-Wistar rats was investigated. All tumors were subcutaneously implanted by inoculating  $10^5$  viable cells of Pollard rat prostatic adenocarcinoma (PA-III) into the right flank of each animal. This tumor model was selected because it is known  
35 to metastasize uniformly and spontaneously from extravascular sites only through ipsilateral lymphatic channels. Because of this tumor's unique, predictable

spread, the contralateral side of the animal could be used as a control. In addition, cancer detection is facilitated with this model because rats with PA-III cells survive beyond forty days after implantation without  
5 evidence of physical impairment.

After forty-two days of observation, Photofrin II (QLT Phototherapeutics, Inc., Vancouver, Canada) was administered intraperitoneally twenty four - forty eight hours prior to surgical exploration in doses ranging from  
10 0.75 - 7.5 mg/kg. Eighteen animals were divided into eight groups and injected with four different concentrations of Photofrin II (7.5 mg/kg, 2.5 mg/kg, 1.5 mg/kg and 0.75 mg/kg). Metastatic detection was performed during abdominal exploration of the renal, para-aortic,  
15 and iliac lymph nodes after laparotomy. The rats were anesthetized with ketamine 40 mg/kg and Xylazine 5.0 mg/kg intraperitoneally, and the abdomens opened through a midline incision. Advancing caudally to cranially, ipsilateral inguinal, iliac, para-aortal and renal lymph  
20 nodes were scanned. The areas were scanned in a contact mode with a hand held fiber along the iliac artery to medial and along the aorta to the para-aortic and renal lymph nodes (50 acquisition points/second, 3-4 detection sites/minute, going back and forth. Otherwise, the number  
25 of sites could increase.)

Nineteen abnormal tissue samples were removed for histological analysis, eleven of which were larger than 5 mm. Laser-induced fluorescence spectroscopy revealed malignancy in all eleven cases. In eight excised tissue  
30 samples with dimensions less than 5 mm, laser-induced fluorescence spectroscopy suggested malignancy, but histology confirmed malignancy in only three samples (see Table I below). Lymph nodes and tissues where tumor was detected were immediately excised for further histologic  
35 examination. The contralateral side was scanned in the same manner and contralateral lymph nodes were excised for histological examination. Fluorescence spectra were also

obtained from the liver, kidney, stomach, skin, muscle and large and small bowel.

Table I

Tissue Samples Identified As Malignant By LIFS (n=19)

5 <u>Tumor Size</u>	<u>Histological Identification</u>	
	<u>Malignant</u>	<u>Normal</u>
> 5mm	11	0
< 5mm	3	5
p< .01 (Fisher's exact test)		

10 FIG. 2 illustrates the time-related spectral curves for cancerous and normal rat tissue after Photofrin II administration. The Y-axis represents fluorescence intensity, the X-axis represents wavelength and the Z-axis represents time. The fluorescence spectrum was  
 15 recorded at every 20th millisecond using a OMA III system while puncturing the targeted rat tissue with the optical biopsy needle. Both the autofluorescence of the tissue and the fluorescence signal of the malignant tissue containing Photofrin II were recorded. The  
 20 autofluorescence peak is located at 540 nm, and a more prominent peak representing metastasis is located at 630 nm. Note that the intensity of the prominent peak at 630 nm increases as the optical biopsy needle is advanced into the metastasis.

25 FIG. 3 illustrates the intensity ratio of Photofrin II to autofluorescence for the Photofrin II distribution in rat muscle, primary tumor (right flank) and abdominal metastasis. The Y-axis represents the Intensity Ratio ( $I_{630 \text{ nm}}/I_{540 \text{ nm}}$ ), the X-axis represents the particular sample  
 30 number tested, and the bars represent the three different areas scanned: the circle illustrating metastasis, the shaded bar illustrating tumor, and the solid bar illustrating normal muscle mass. The intensity ratio of Photofrin II fluorescence at 630 nm and tissue  
 35 autofluorescence at 540 nm was calculated for each of 20

rats to eliminate interference from background fluorescence due to intrinsic porphyrins in cancerous tissue and limited porphyrin uptake in normal tissue. Rats No. 1 and No. 18 were used as controls. The  
5 intensity ratio was calculated from fluorescence produced by four different doses of Photofrin II (0.75 mg/kg, 1.5 mg/kg, 2.5 mg/kg and 7.5 mg/kg) and two post-injection times of 24 and 48 hours. The prominent peaks for metastasis demonstrates a consistent and significantly  
10 higher uptake of Photofrin II by metastasis than by primary tumor for all post injection times.

The graph in FIG. 4 uses the same intensity ratio of Photofrin II fluorescence to autofluorescence to demonstrate the optimum time for Photofrin II accumulation  
15 in muscle, primary tumor, and metastasis after injection. Here again, the Y-axis represents the Intensity Ratio, but the X-axis represents the two post-injection times of 24 hours for the 3 bars left of center, and 48 hours for the three bars right of center. The three bars represent the  
20 three different areas of the body where the intensity ratio was monitored: the diagonal shading representing metastasis, the dotted shading representing tumor and the solid bar representing normal muscle mass. Signals at the site of the primary tumor and lymph nodes with metastasis  
25 show a higher ratio at 48 hours than at 24 hours after Photofrin II administration. In contrast, the ratio at the thigh muscle site was higher at 24 hours than at 48 hours. (See also Tables II and III below).



Table II

Intensity Ratio:  $I_{630nm}/I_{540nm}$  vs.Time After Administration of Photophrin II

Injection dose:		1.5 mg/kg
5	Time after administration:	24 hrs; n=2
	Tissue	Intensity Ratio ( $I_{630nm}/I_{540nm}$ )
	Muscle	$0.5 \pm 0.1$
	Tumor	$1.0 \pm 0.1$
10	Metastases	$3.3 \pm 0.1$
	Time after administration:	48 hrs; n=2
	Tissue	Intensity Ratio ( $I_{630nm}/I_{540nm}$ )
	Muscle	$0.22 \pm 0.15$
15	Tumor	$2.55 \pm 1.5$
	Metastases	$5.00 \pm 0.1$

Table III

Intensity Ratio:  $I_{630nm}/I_{540nm}$  vs.

## Photofrin II Administration Dose

20	<u>Time After Administration: 48 Hours</u>		
	<u>Tissue</u>	<u>Photofrin II Dose (mg/kg)</u>	
		<u>0.75 (n=3)</u>	<u>1.5 (n=2)</u>
	Muscle	$0.34 \pm 0.06$	$0.22 \pm 0.15$
	Tumor	$0.80 \pm 0.7$	$2.55 \pm 1.5$
25	Metastases	$2.5 \pm 0.8$	$5.00 \pm 0.1$
		<u>2.5 (n=2)</u>	<u>7.5 (n=2)</u>
	Muscle	$0.36 \pm 0.36$	$0.48 \pm 0.24$
	Tumor	$1.82 \pm 1.9$	$3.64 \pm 3.65$
	Metastases	$5.2 \pm 3.36$	$4.64 \pm 2.99$

30 FIG. 5 demonstrates the optimum Photofrin II dosage range for metastasis detection using the same fluorescence intensity ratio of Photofrin II fluorescence to autofluorescence for different doses 48 hours after administration. The Y-axis represents the Intensity Ratio

35 while the X-axis represents the four different Photofrin

II injection doses tested. The first three bars from the left side of the graph illustrate the Intensity Ratio at a dose of 7.5 mg/kg, the second three bars from the left illustrate the Intensity Ratio at a dose of 2.5 mg/kg, the  
5 third three bars illustrate the Intensity Ratio at a dose of 1.5 mg/kg, while the last three bars illustrate the Intensity Ratio at a dose of 0.75 mg/kg. Again, the bars with diagonal shading represent metastasis, the bars with dotted shading represent tumor and the solid bars  
10 represent normal muscle tissue. The bar graph illustrates no significant difference in the metastasis intensity ratio for Photofrin II doses of 1.25 mg/kg, 2.5 mg/kg and 7.5 mg/kg. Although the metastasis intensity ratio at 0.75 mg/kg was somewhat lower than at the other three  
15 dosages, it was still useful to distinguish metastatic tissue from primary tumor.

In summary, it has been discovered that when the ratio of intensity of Photofrin II fluorescence to autofluorescence is observed ( $I_{630\text{ nm}}/I_{540\text{ nm}}$ ), there is a  
20 consistent and significant difference between metastasis and primary tumor as well as metastasis and normal or benign tissue. The ratio readings for metastasis are consistently higher, by a significant margin, than for primary tumor and normal tissue. Based on this knowledge,  
25 the detection and subsequent biopsy of disseminated metastatic cancer can be made for cancer diagnosis and treatment.

Claims

1. A method for the detection and biopsy of metastatic cancer comprising:

- a. administering an effective amount of a  
5 photosensitizing agent capable of light-induced fluorescence,
- b. exciting a tissue to be examined with a beam of light from a light source,
- c. comparing the fluorescence emitted from the  
10 examined tissue with the fluorescence emitted from tissue whose condition is known, and
- d. excising for histological examination tissue that displays a fluorescence pattern different from tissue whose condition is known using a biopsy blade integrated  
15 with the light source.

2. A method for the detection and biopsy of metastatic cancer comprising:

- a. administering an effective amount of a  
20 photosensitizing agent capable of light-induced fluorescence,
- b. exciting a tissue to be examined with a beam of light from a light source,
- c. observing the fluorescence emitted from a tissue to be examined and tissue whose condition is known each at  
25 two wavelengths,
- d. determining if the tissue is cancerous in accordance with said measurements, and
- e. excising for histological examination the cancerous tissue using a biopsy blade integrated with the  
30 light source.

3. A method for the detection and biopsy of metastatic cancer comprising:

- a. administering an effective amount of a  
35 photosensitizing agent capable of light-induced fluorescence,

b. exciting a tissue to be examined with a beam of light from a light source,

c. observing the fluorescence emitted from a tissue to be examined and tissue whose condition is known each at  
5 two wavelengths,

d. determining if the tissue is cancerous in accordance with said measurements,

e. determining the depth of cancer involvement in the affected tissue using a biopsy blade integrated with  
10 the light source, and

f. excising for histological examination the cancerous tissue using the biopsy blade.

4. A method for the detection and biopsy of metastatic cancer comprising:

15 a. administering an effective amount of a photosensitizing agent capable of light-induced fluorescence,

b. exciting a tissue to be examined with a beam of light from a light source that is at least substantially  
20 monochromatic and/or incoherent light filtered to a specific wavelength,

c. comparing the fluorescence emitted from the examined tissue with the fluorescence emitted from tissue whose condition is known, and

25 d. excising for histological examination tissue that displays a fluorescence pattern different from tissue whose condition is known using a biopsy blade integrated with the light source.

5. A method for the detection and biopsy of  
30 metastatic cancer comprising:

a. administering an effective amount of a photosensitizing agent capable of light-induced fluorescence,

b. exciting a tissue to be examined with a beam of light that is at least substantially monochromatic and/or incoherent light filtered to a specific wavelength,

c. observing the fluorescence emitted from a tissue  
5 to be examined and tissue whose condition is known each at two wavelengths,

d. determining if the tissue is cancerous in accordance with said measurements, and

e. excising for histological examination the  
10 cancerous tissue using a biopsy blade integrated with the light source.

6. A method for the detection and biopsy of metastatic cancer comprising:

a. administering an effective amount of a  
15 photosensitizing agent capable of light-induced fluorescence,

b. exciting a tissue to be examined with a beam of light that is at least substantially monochromatic and/or incoherent light filtered to a specific wavelength,

20 c. observing the fluorescence emitted from a tissue to be examined and tissue whose condition is known each at two wavelengths,

d. determining if the tissue is cancerous in accordance with said measurements,

25 e. determining the depth of cancer involvement in the affected tissue using a biopsy blade integrated with the light source, and

f. excising for histological examination the cancerous tissue using the biopsy blade.

30 7. The method of claim 6 and wherein determining if cancerous tissue is present comprises determining the ratio of intensities at two wavelengths and then comparing the ratio of intensities at the same wavelengths for primary tumor and normal tissue.

8. The method of claim 6 and wherein determining if cancerous tissue is present includes producing a signal corresponding to the ratio between the intensities at two wavelengths and then displaying said signal.

5 9. A method for the detection and biopsy of metastatic cancer comprising:

a. administering an effective amount of a photosensitizing agent capable of light-induced fluorescence,

10 b. exciting a tissue to be examined with a beam of monochromatic light and/or incoherent light filtered to a specific wavelength from a light source,

c. generating a fluorescence spectrum of light emitted by the tissue,

15 d. determining the ratio of intensities at two wavelengths for the examined tissue and then comparing the ratio of intensities at the same wavelengths for primary tumor and normal tissue,

e. determining the depth of cancer involvement in  
20 the affected tissue using a biopsy blade integrated with the light source, and

f. excising for histological examination tissue that displays a fluorescence pattern different from that of known tissue using the biopsy blade.

25 10. The method of claim 9 and wherein the two wavelengths from which fluorescence is observed are 630 nm and 540 nm.

11. The method of claim 9 and wherein the photosensitizing agent is selectively retained by  
30 cancerous tissue.

12. The method of claim 9 and wherein an effective amount of the photosensitizing agent is administered by injection.



13. The method of claim 9 and wherein determining the depth of cancer involvement of the affected tissue comprises using a calibrated biopsy needle.

14. An apparatus for the detection and biopsy of  
5 metastatic cancer comprising:

a. a light source capable of inducing fluorescence by photosensitizing agents selectively retained by cancerous tissue, and

b. a blade to remove suspected cancerous tissue,  
10 upon which blade the light source is integrated.

15. An apparatus for the detection and biopsy of metastatic cancer comprising:

a. a light source emitting substantially monochromatic light and/or incoherent light filtered to a  
15 specific wavelength capable of inducing fluorescence by photosensitizing agents selectively retained by cancerous tissue, and

b. a blade to remove suspected cancerous tissue, upon which blade the light source is integrated.

20 16. The apparatus of claim 15 and wherein the light source comprises multiple or single fiber arrays with a core diameter from about 400 microns.

17. The apparatus of claim 15 and wherein the blade is calibrated to measure the depth of cancer involvement  
25 in a tissue.

1/5

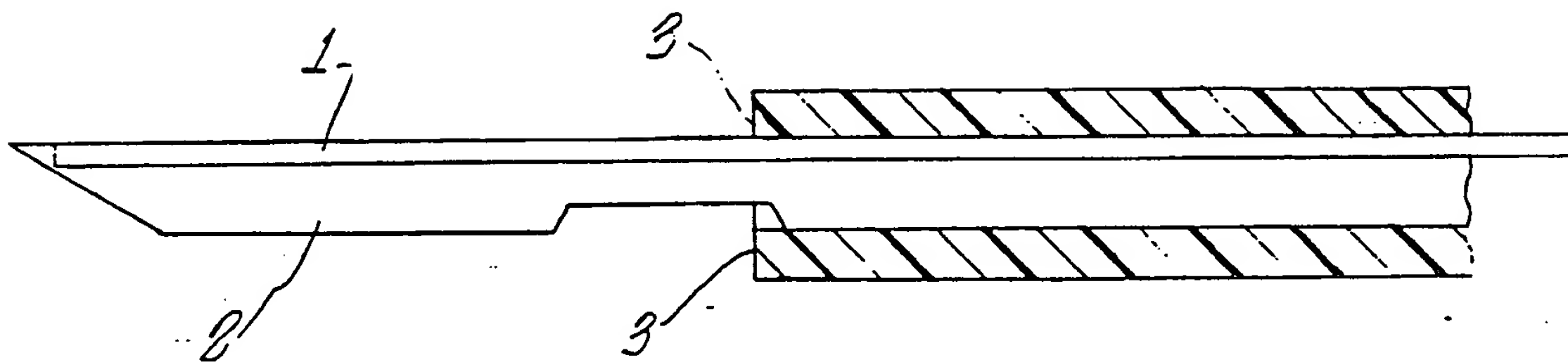


FIG. 1A.

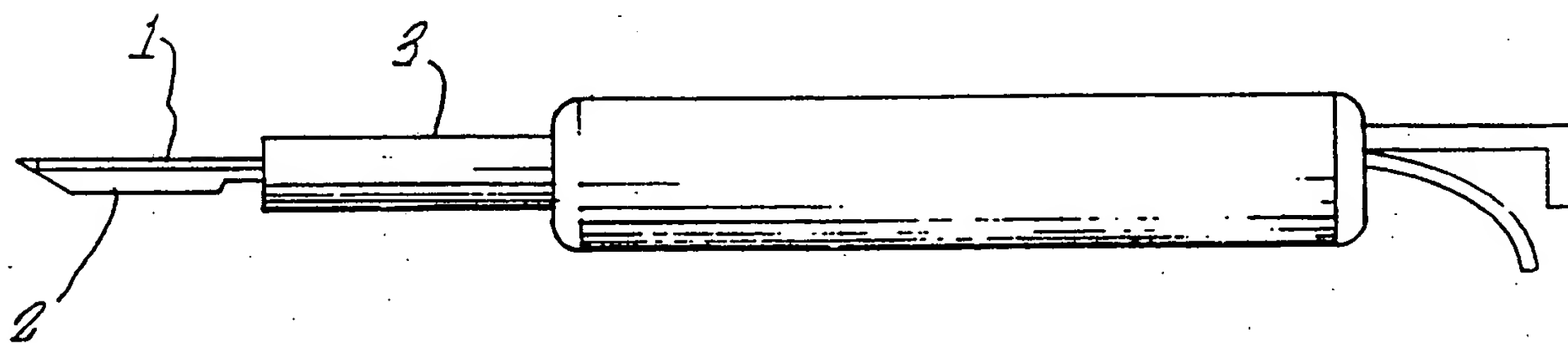
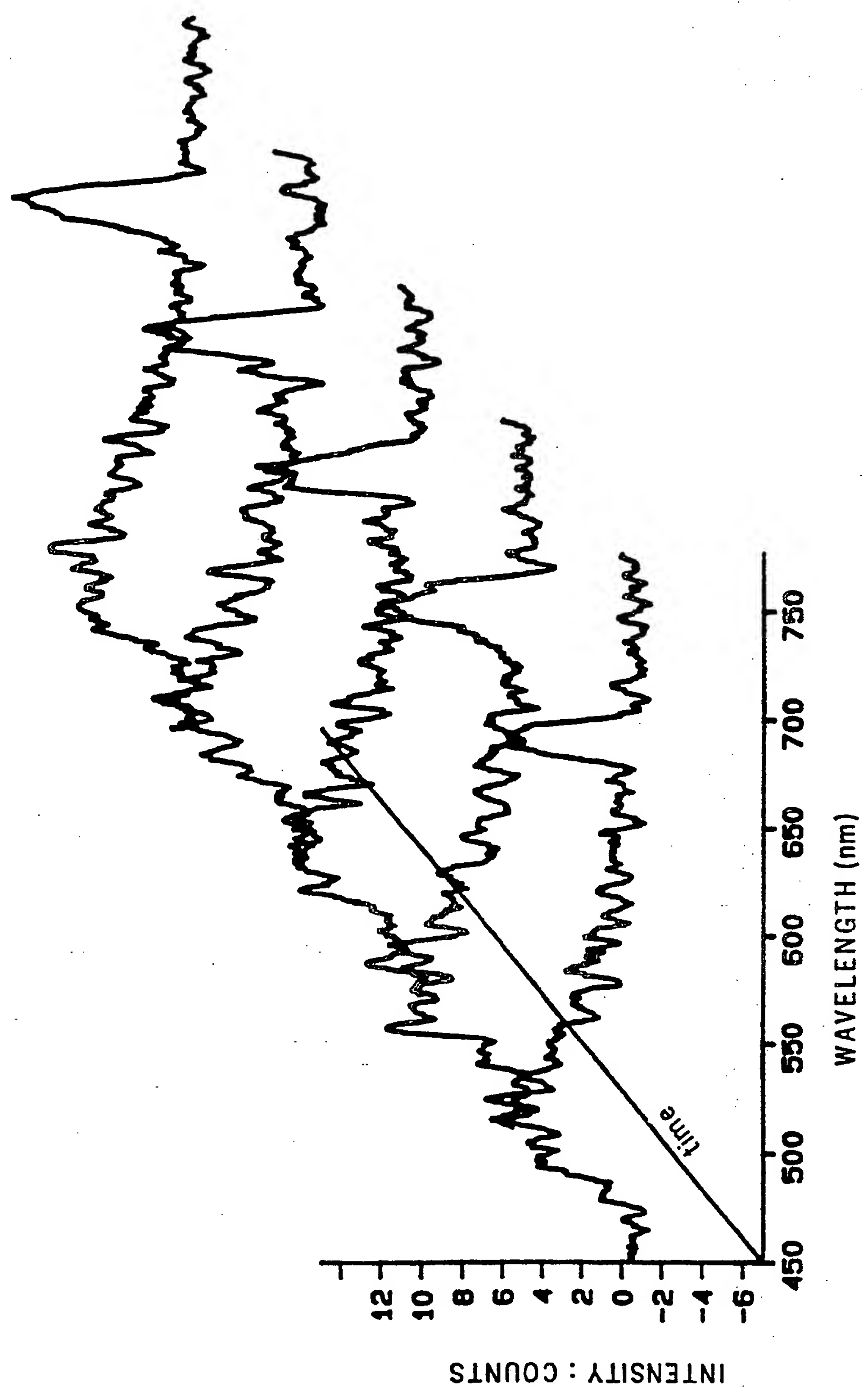


FIG. 1B.

Fig. 2-



3/5

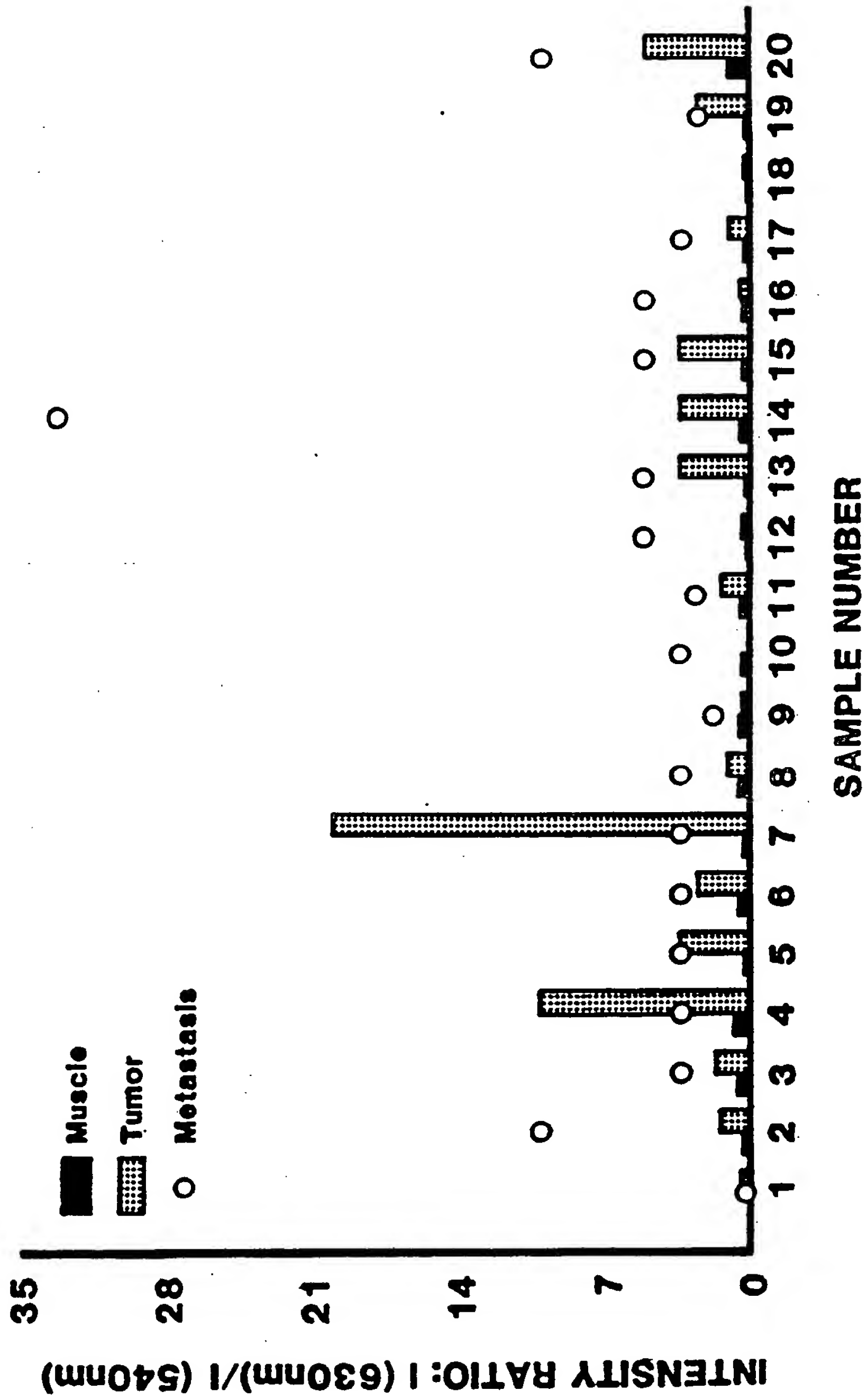


Fig. 3-

Fig. 4.

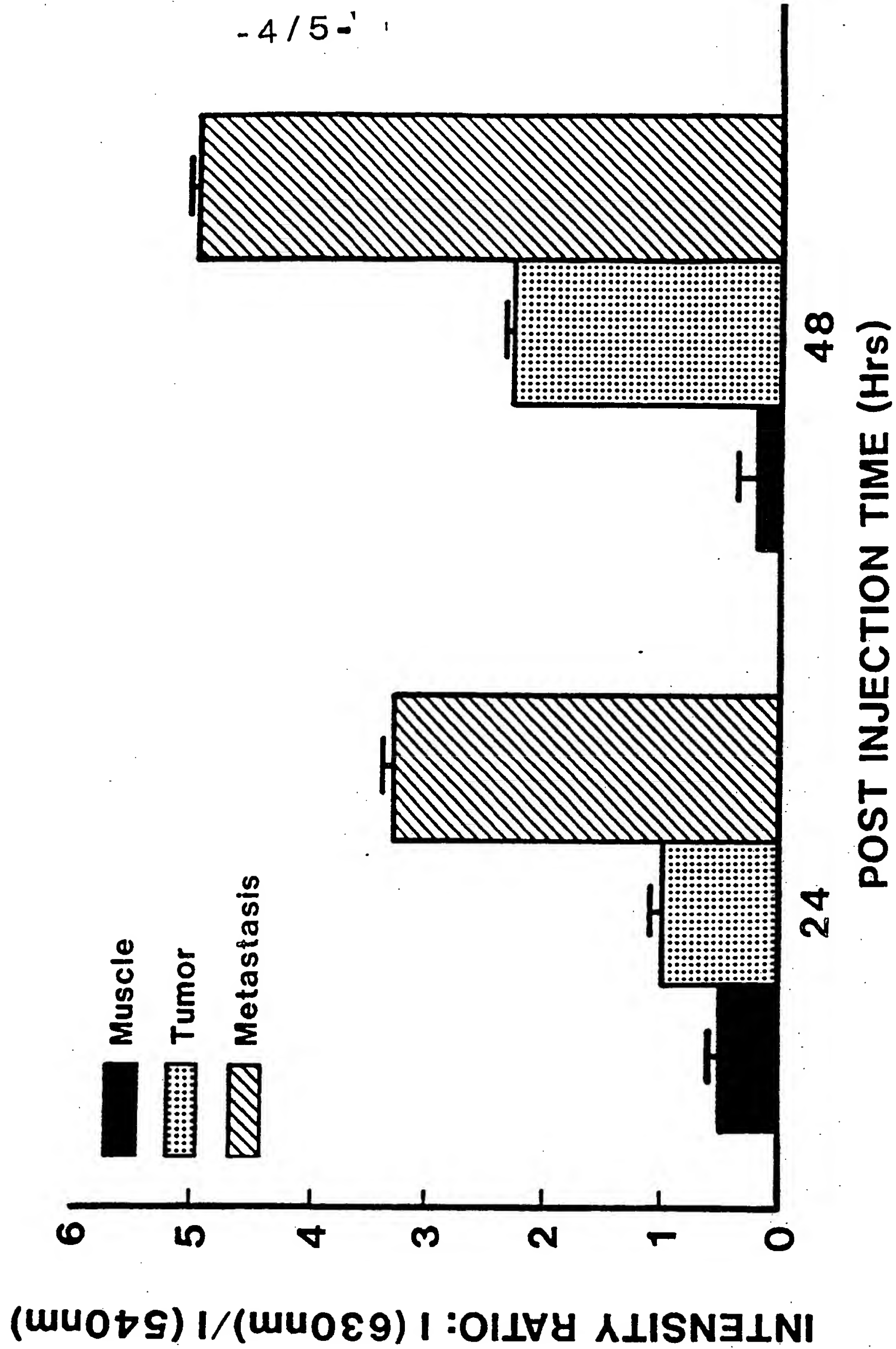
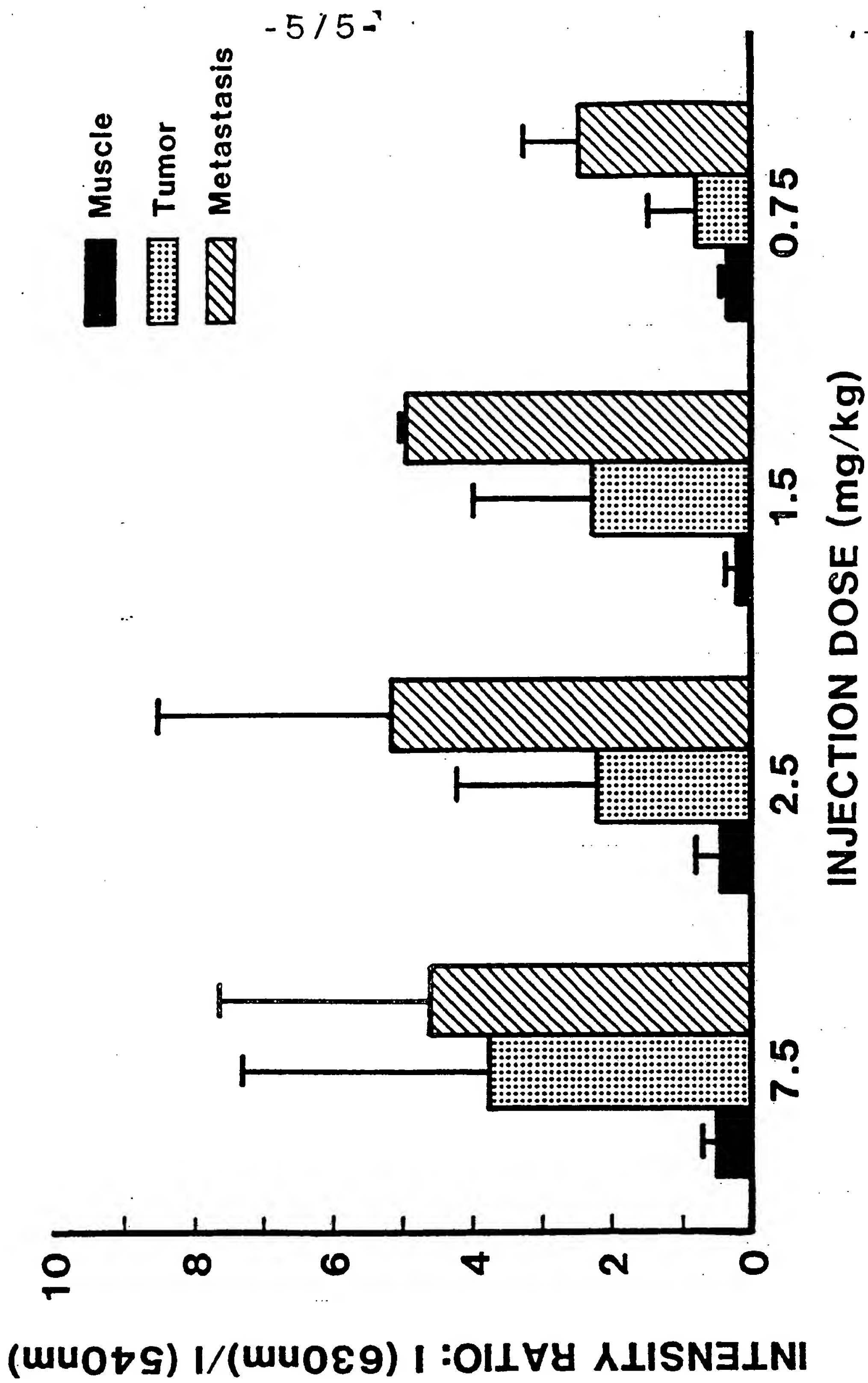


FIG 5





# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/02042

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC		
U.S. CL. 128/634      IPC(5): A61B 5/00		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
U.S.	128/633, 634, 664, 665  606/2, 13, 14, 15	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	US,A, 4,140,109 (Savic et al) 20 February 1979 See the claims.	13,17
Y	US,A, 4,273,127 (Auth et al) 16 June 1981 See the cover figure; elements 12,14,20,22.	1-17
Y	US,A, 4,556,057 (Hiruma et al) 03 December 1985 See the whole specification.	1-17
Y	US,A, 4,887,600 (Watson et al.) 19 December 1989 See columns 1 and 3 of the specification and claim 1	16
YP	US,A, 5,074,306 (Green et al.) 24 December 1991 See the abstract and the whole specification.	1-13
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>14</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
28 May 1992		7 JUL 1992
International Searching Authority		Signature of Authorized Officer
ISA/US		A. Paul Zuttarelli